

## Pressing Issues in the Health Effects Assessment of Chemical Substances

YASUNOBU AOKI (*Affiliated Fellow*)

### 1 Introduction: Hazard and risk assessments of chemical substances

It's anybody's guess how many different chemicals are currently used in items such as chemical products, solvents and synthetic resins. Estimates abound, but the number of registered or listed chemicals suggests that some 100,000 chemicals are in use today, of which as many as 50,000 are on the Japanese market.

It's not long since most these chemicals<sup>\*1</sup> came into widespread use - the latter half of the 20th century saw a rise in their mass production, with a variety of synthetic processes developed. For this reason, some "dream" chemical substances with useful properties were found to be hazardous only after they had become ubiquitous. For instance, trichloroethylene and tetrachloroethylene - chemicals once termed "dream detergents" - were used in large quantities as degreasing/cleaning agents for precision instruments and semiconductors. Because they were shown to be carcinogenic in animal experiments, however, their use was restricted in many countries including Japan. Fortunately, both trichloroethylene and tetrachloroethylene were shown to be hazardous before they began to have an impact on human health. Much has to be done to assess the hazardous properties of many other chemicals.

As we enter the 21st century, there has been a growing trend to assess the potential risks of technologies and products developed in the 20th century before making use of them. For instance, the Cartagena Protocol, which was concluded in 2003, regulates the import of genetically modified organisms; their potential risks should be

assessed to conserve biodiversity and minimize impacts on human health. Underlying these arrangements is the concept of precautionary approach to control agents of unknown hazard.

Any secondary hazard should be quantified to assess the potential risks of new technologies and products. One of the reasons why the health effects of radiation are assessed strictly is that an enormous amount of biological and medical research has shed light on the relationship between the dosage of radiation and the associated hazard. To assess the risks of chemicals, therefore, there is a need to quantify their hazardous properties, particularly the probability of an impact on human health. The mechanism used to assess the hazardous properties of chemicals is worth noting as a model for systematically acquiring the information required for risk assessments.

### 2 Why should the hazardous properties of chemical substances be assessed in advance?

In Japan, the hazardous properties of chemical substances are assessed in accordance with the "Law concerning the Evaluation of Chemical Substances and Regulation of their Manufacture etc.", which was enacted in 1973 (hereinafter referred to as "the law") in the wake of the Yusho incident - a PCB poisoning that occurred primarily in northern part of Kyushu area in 1968. This incident revealed that environmental pollution by PCB was widespread in Japan and its accumulation in organisms was more serious than expected, a situation that raised public awareness of health effects due to exposure to hazardous chemicals. The law is one of the first of

its kind in the world to regulate production and import of chemical substances in view of their potential impacts on human health. It's safe to say that Japan led the way in taking safety measures against chemical substances when it instituted the law. We learned some important lessons from our experience in industrial pollution, which may have prompted us to take specific action.

The hazardous properties of chemicals should be assessed in advance to minimize environmental pollution and prevent any possible impact on human health and ecosystems. It usually takes some time before the hazardous properties of chemical substances are well understood by the public, and appropriate safety measures are in place. In the U.S., for instance, commercial production of PCB began in the 1930s, but it was not until the latter half of the 1950s that reproductive failure was found in minks fed with PCB-contaminated fish. In Japan, meanwhile, a total of some 60,000 tons of PCB was produced in the 18 years from 1954 (when commercial production of PCB started) to 1972 (when production was discontinued due to the Yusho incident)<sup>[1]</sup>. The pollution was already widespread when PCB proved to be hazardous. Cases abound in Japan where soil and groundwater are polluted with heavy metals and persistent chemicals such as PCB and trichloroethylene<sup>[2]</sup> (see Figure 1). The cost of removing pollutants already dispersed in the environment is extremely high, and could

have been avoided if appropriate antipollution measures had been in place based on an understanding of their hazardous properties.

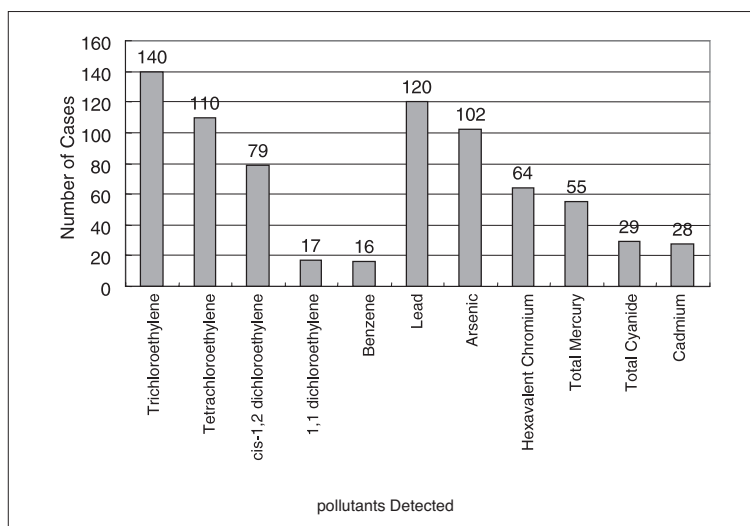
Assessing the hazardous properties of chemicals is essential to assessing the risks of exposing humans to them, and this is becoming increasingly important. Specifically, risk assessment of chemicals refers to quantitative assessment of the health effects on humans and wildlife exposed to them in the environment. Fundamental to risk assessments of exposure to chemical substances is to identify 1) the toxicity of the chemical substances concerned, and 2) the possible amount of which humans and other organisms are exposed to them. In other words, the degree of toxicity multiplied by the amount of exposure equals risk<sup>[3]</sup>.

Generally speaking, hazard assessments of chemicals mean identification of the toxicity of the chemicals concerned. As there are still a number of chemical substances whose hazardous properties have yet to be determined, assessing the toxicity of each individual chemical is essential to appropriate risk management - e.g., risk assessments of a variety of chemicals, and risk abatement measures.

### 3 Status of hazard assessment of chemical substances

Chemicals can be broadly classified in two categories for management purposes. Those

**Figure 1** : Number of cases where concentration of pollutants in soil exceeded standards



Source: Outline of the Survey of Soil Contamination, Antipollution Measures, etc., by the Ministry of the Environment (2001)

whose production and import started after the enforcement of the law (1973) are termed “new chemical substances.” The businesses concerned are obliged to conduct the tests for hazard assessment, the results of which are reviewed jointly by the Ministry of Economy, Trade and Industry, the Ministry of Health, Labour and Welfare, and the Ministry of the Environment to control the production and import of potentially hazardous chemical substances. The goal is to reduce their release to the environment and human exposure. Other chemical substances, whose production and import started before the enforcement of the law, are termed “existing chemical substances”; their hazardous properties are being examined by the government so that they are included in the framework of chemical substance management (see Figure 2).

Businesses dealing with existing chemicals such as benzene, the hazardous properties of which have yet to be determined, are using their accumulated knowledge to reduce the release of those chemicals into the environment.

Other countries are taking similar measures to control potentially hazardous chemicals. The hazardous properties of new chemicals are determined by governments (based on data provided by businesses), while those of existing chemicals are reviewed independently by each

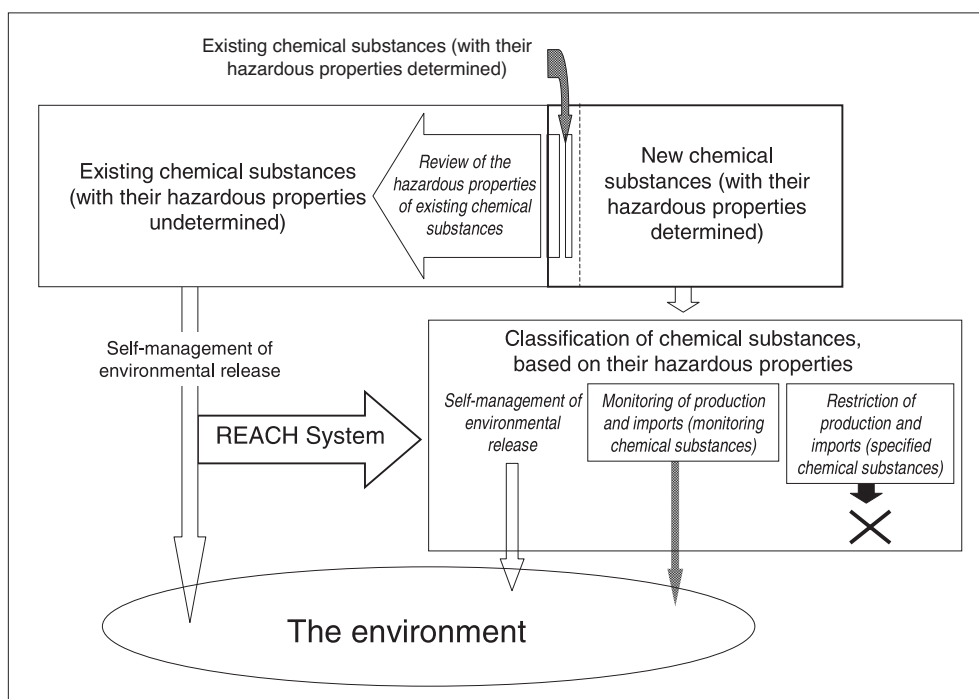
country.

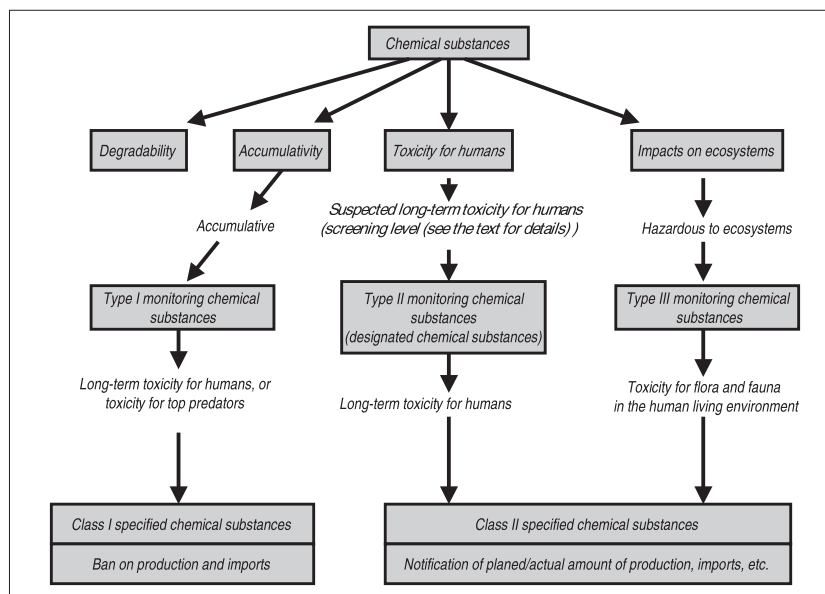
### 3-1 Assessment of the hazardous properties of new chemical substances in Japan

Under the previous version of the Law concerning the Evaluation of Chemical Substances and Regulation of their Manufacture etc., the hazardous properties of new chemicals were determined based on their 1) biodegradability, 2) bioaccumulativity and 3) the results of toxicity screening tests. With the law revised in 2003, the results of ecological toxicity tests will be added to the existing criteria in fiscal 2004 (refer to page 41 of this bulletin<sup>(4)</sup>).

The toxicity of chemicals should be determined by the results of a variety of long-term tests such as those for chronic toxicity, carcinogenicity and teratogenicity - an extremely costly procedure. Thus two types of convenient tests (screening toxicity tests) complying with the OECD Directive (1983) will be used to examine the possible long-term toxicity of chemical substances to humans. One is a genotoxicity test, which uses bacteria and cultured cells to examine the possible carcinogenicity of chemical substances. The other is what is called a “repeated-dose 28-day oral toxicity test,” which administers chemical substances to rats for 28 consecutive days to determine the maximum

Figure 2: Chemical substance management system



**Figure 3** : Outline of the revised Chemical Substances Control Law (2003)

Subjects relevant to health effects are presented in italic

dose that does not produce pathological changes. The degree of toxicity is determined, taking into consideration the results of these tests. Both businesses and reviewers are making significant efforts to conduct the screening toxicity tests.

The hazardous properties of chemical substances are determined, based on the results of tests conducted by businesses, and those chemicals are monitored and controlled in accordance with their hazardous properties. With the law revised in 2003, potentially hazardous chemicals will be classified into three categories (Type I-III monitoring chemical substances), based on the nature of their hazardous properties. The actual amount of their production and import are to be reported. On the other hand, chemical substances to be monitored, whose long-term toxicity to humans or wildlife has been identified, will be classified into two categories (Class I-II specified chemical substance), and they will either be banned or regulated (see Figure 3). A total of 4,322 chemicals were reported as “new chemical substances” during April 1987 and December 2002 (the period when the conventional examination system was in place), of which 821 (or about 19% of the total) were classified as “designated chemical substances”<sup>[5]</sup> (equivalent to “Type-II monitoring chemical

substance,” under the revised law).

### 3-2 Assessment of the hazardous properties of new chemical substances in other countries

In accordance with the 7th Council Directive (1992) on the classification, packaging and labeling of dangerous substances, hazard assessments are in place in the EU for any chemical substance that is produced in or imported into the region in quantities greater than one ton/year, following procedures similar to those in Japan. Specifically, chemicals determined to be hazardous should be labeled according to their hazard levels before going on the market, and those involving significant risks should be closely monitored and regulated.

In the case of the U.S., meanwhile, chemicals are regulated in accordance with the Toxic Substance Control Act (TSCA). Any data concerning the hazard of chemical substances that have been produced in or imported into the country since 1980 in quantities greater than 10 ton/year should be reported to the Environmental Protection Agency (EPA). Chemicals determined to be hazardous should be handled and disposed of in accordance with the procedures specified by the Act.

## 4 Reactive hazard assessments of existing chemical substances

### 4-1 Status in Japan

About 20,000 items are registered as “existing chemical substances” in Japan, most of which have been in use. The Ministry of Economy, Trade and Industry has jurisdiction over biodegradability and bioaccumulativity tests, and the Ministry of Health, Labour and Welfare, over toxicity screening tests, the results of which serve as the basis for reclassifying some of the existing chemical substances as designated chemical substances (equivalent to “Type-II monitoring chemical substance,” under the revised law). Beginning fiscal 2004, the Ministry of the Environment will take charge of ecotoxicity tests to designate “Type-III monitoring chemical substance.”

Yet little progress is being made in hazard assessment of existing chemicals. In fact, of some 20,000 registered “existing chemical substances,” a total of only 1,377 items have been tested for their biodegradability and bioaccumulativity by 2002, while about 200 items have undergone toxicity screening tests, and 134 items have been classified as “designated chemical substances<sup>[5]</sup>.”

### 4-2 Status in EU and the U.S.<sup>[6,7,8]</sup>

As many as 100,000 items registered as “existing chemical substances” were produced in or imported into the EU member states before 1981; those involving more than 1,000 ton/year of production or import have been tested for their toxicity and possible impacts on ecosystems. The results of these tests are in the “International Uniform Chemical Information Database (IUCLID),” which has information on the hazard of various chemical substances. Risk assessments of existing chemical substances have been underway since 1994 based on this database. The results: 141 items are designated as “priority chemical substances” and a risk report was drafted in April 2002 for 88 items, with risk-reduction measures required for 45 items.

In the U.S., businesses are conducted to assess the hazardous properties of priority existing chemical substances (more than 500 items)

designated by EPA in accordance with TSCA. However, only 80 items have been examined so far - a delay due to the need to test various toxicity such as chronic toxicity and carcinogenic tests. According to the TSCA inventory, the total of new chemical substances and existing chemical substances is approaching 70,000, with data on their hazard available for public review.

### 4-3 OECD program to investigate high production volume (HPV) chemicals<sup>[9]</sup> (OECD HPV Program)

Chemicals being produced in large quantities have a greater likelihood of being released into the environment, thus increasing the possibility of human exposure. The OECD published a rule in 1992 for HPV (High Production Volume) chemical substances involving more than 1,000 ton/year of production in a given country. Each country is to prepare a report on the hazardous properties of the HPV chemicals concerned, collecting data on their physico-chemical qualities, environmental fates, ecotoxicities and toxicities (repeated-dose 28-day oral toxicity etc.) - a minimum requirement for hazard assessments. A program to investigate HPV chemicals (OECD HPV Program) was launched in 1999, with the aim of assessing 1,000 items out of some 5,200 on the list by 2004. However, only 371 items had been completed as of 2003<sup>[10]</sup>.

The U.S. also launched its own initiative, termed the “HPV Challenge Program”, in 1998 sharing relevant information with the OECD<sup>[7,11]</sup>. The HPV chemicals to be investigated in this program (i.e., organic compounds that are produced in or imported into the country in quantities greater than 454 ton/year; polymers excluded) totaled some 2,800 items, which originally accounted for 11% of the items listed on the TSCA inventory, or 95% of the total production of chemical substances in the country. However, complete data on health and environmental effects are available for only 7% of the HPV chemicals to be investigated, which clearly suggests the need to prioritize the hazard assessment of HPV chemicals.

Of 1,606 chemicals, knowledge of whose production in or import into Japan is made available to the public, 798 items are produced in



or imported into the country in quantities greater than 1,000 ton/year (see Table 1). The production and import of all the chemicals on the market needs to be made public to promote hazard and risk assessment.

## 5 Trends in the management of new chemical substances in the EU: the REACH System

The European Commission proposed the “Strategy for a Future Chemicals Policy” in February 2001. The underlying goal of this strategy is to avoid using chemicals with unknown toxicity. To this end, 1) new chemical substances and existing chemical substances will be regulated, based on the same standards (see Figure 2), and 2) the burden of proof regarding the safety of chemical substances will be transferred from the governments of the member states to the industries concerned (including not only chemical manufacturers and importers but also users). There is also a move afoot to shift the entire responsibility for controlling chemical substances from government to industry<sup>[7,12]</sup>.

The REACH (Regulation, Evaluation and Authorization of Chemicals) System is a framework for implementing the proposed strategy. Regardless of the categories of chemical substances, it stipulates that 1) data on the toxicity and the amount of human/environmental exposure of chemicals be registered with the system by businesses, 2) the hazardous properties of chemicals produced or imported in large quantities be evaluated thoroughly, and 3) highly hazardous chemicals in terms of their carcinogenicity and teratogenicity be authorized prior to use (These chemicals, in principle, should be replaced by safer chemicals. Their production or import will be banned unless businesses can prove that they pose a negligible risk or have no substitutes available.)

The proposed strategy is under consideration by the European Parliament. It is indeed an epoch-making initiative to prevent the release of hazardous chemical substances into the environment, though there are many who disapprove of this attempt, asserting that “it may impose too much of a burden on industry.” It is

**Table 1** :Survey of production and imports of chemical substances in fiscal 2001

Production/Imports (ton)	Number of Items
1 – 10	12
10 – 100	239
100 – 1,000	557
1,000 – 10,000	446
10,000 – 100,000	216
100,000 – 1,000,000	99
1,000,000 – 10,000,000	34
10,000,000 or more	3

Source: Chemical Management Policy Division, Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry

worth noting, however, that the environmental movement in Europe has become so powerful that it is beginning to have much sway over industrial policies in the region.

## 6 Measures to complement efficient hazard assessment of existing chemical substances

The majority of existing chemicals have yet to be assessed for their hazardous properties, both at home and abroad - a situation due in part to the considerable amount of time and cost involved in toxicity tests<sup>2</sup>.

It is ultimately impossible to assess the toxicity of chemical substances without animal experiments. Meanwhile, it would be ideal if all the chemicals on the market could be assessed, but this is also impossible in view of the cost and time involved. While hazard assessments are currently prioritized for HPV chemicals, potentially highly toxic chemical substances should be screened out and assessed for their toxicity as a means to prevent adverse health effects from exposure to chemicals, with the first priority being to collect data efficiently. A simple, quick and easy way to predict the toxicity of chemical substances should thus be developed to determine these priorities.

Risks associated with chemicals can be reduced by preventing the release of hazardous ones into the environment, as well as preventing the exposure of humans and wildlife. In this context, chemicals that are detected frequently

and in high concentrations in the air and water should be assessed for their hazardous properties, regardless of their production or import volume.

It is also essential that the domestic institutional framework be improved to promote hazard assessments of existing chemical substances - which should be based on more reliable data obtained through overseas HPV programs, rather than on data collected in Japan. OECD, EU, the U.S. and Japan have yet to share their databases on toxicity with one another. These databases should be unified to promote hazard assessments on a worldwide basis.

Promoting efficiency in obtaining toxicity data is important not only with respect to existing chemicals. With the revised law in place, chemicals produced or imported in quantities smaller than 10 ton/year (the previous threshold: one ton/year) are classified as "chemical substances with low volume manufacturing/import," most of which are exempted from toxicity tests. New chemical substances, however, include a great many chemicals that have been in use for a relatively short time. For instance, the variety of designated chemical substances containing fluorine has increased dramatically in recent years. The number of items registered increased from only a handful a year to some 50 in 2000 and 2001 combined. As part of the precautionary approaches for new chemical substances, which are expected to increase further, voluntary efforts should be encouraged as necessary to assess the hazardous properties of "chemical substances with low volume manufacturing/import" that are potentially highly toxic.

## 7 Status of and prospects for the development of toxicity prediction methods

The following two methods are considered promising ways to predict the toxicity of chemical substances:

### 7-1 QSAR (Quantitative Structure Activity Relationship)

This method involves predicting the toxicity of new chemicals, based on a comparison

of their toxicity levels, chemical structures and physico-chemical qualities with those of known chemicals. In compliance with TSCA, QSAR model termed ECOSAR has been used to predict the ecotoxicity for about 10 years. Specifically, chemicals are classified into 59 categories, and the regression function between logKow (the octanol-water partition coefficient, used to describe the lipophilic or hydrophobic properties of chemical substances) and toxicity (concentration at which 50% of the test organisms are expected to die) is used to predict the toxicity of target chemical substances.

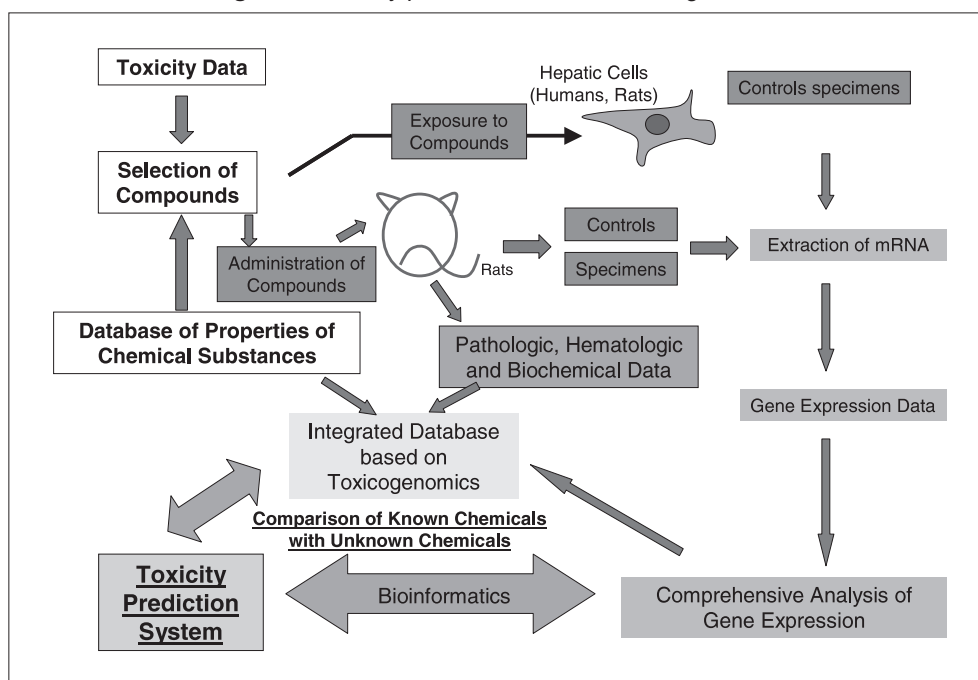
Other toxicity tests such as those for carcinogenicity are also expected to adopt QSAR[13]. EU's REACH System is following suit in an effort to promote hazard assessments.

### 7-2 Toxicogenomics

This method predicts the toxicity of chemical substances using DNA micro array technology. When administered hazardous chemical substances, animals or their cultured cells exhibit certain responses: either increases or decreases in the expression levels of particular genes. With an estimated 10,000 to 20,000 genes to be observed, each of which may respond specifically to the chemicals administered, the changes in gene expression profiles go into a database from which the carcinogenicity of unknown chemicals can be predicted. Specifically, the gene expression profiles of carcinogen are compared with those of other chemicals of unknown carcinogenicity (see Figure 4).

This prediction method, when commercialized, is expected to dramatically improve the efficiency of toxicity tests, as it requires only about one-tenth the time and one-hundredth the cost of conventional methods for predicting the carcinogenicity of a chemical substance.

In the U.S., the National Institute of Environmental Health Sciences, an affiliate of the National Institutes of Health, created the National Center for Toxicogenomics (NCT) in September 2000, which is working on a comprehensive database of gene expression due to exposure to chemical substances[14]. Its "Concept Statement" stipulates that the mission of the NCT is to "use and promote toxicogenomics as a means to guide

**Figure 4 : Toxicity prediction based on toxicogenomics**

Source: Website of the National Institute of Health Sciences

federal agencies and legislators in developing guidelines and laws that regulate the levels of various chemicals in the environment.” On the domestic front, meanwhile, toxicogenomics projects are underway, sponsored by the National Institute of Health Sciences, the Ministry of Economy, Trade and Industry, etc. Expectations are growing for the commercialization of toxicogenomics-based toxicity prediction in five year’s time.

The toxicity prediction methods to be developed next should focus on possible impacts of chemical substances on the offspring in the next generation. There have been concerns over the teratogenicity of chemicals (i.e., the potential to cause birth defects) and their detrimental effects on the nervous system of the fetus. Endocrine-disturbing chemicals also threaten to have possible impacts on the offspring in the next generation. At the present level of technology, however, these impacts can be determined only through animal experiments - a situation that demonstrates the need to develop new toxicity prediction methods.

The development of genetically modified animals is also underway in an effort to come up with tools that would predict the carcinogenicity of chemical substances with short-term tests. When applied systematically in the early stages

of the development of new chemicals and pharmaceuticals, these toxicity prediction methods could make it possible to examine products from the viewpoint of their benefits and safety - which will open up the way to safer chemical substances.

## 8 Conclusion

A great many chemical substances were developed in the 20th century. Exposure to chemical substances previously nonexistent in nature, and their accumulation in human body, were both unprecedented in human history, and hence much remains to be seen about how they impact humans and wildlife. Research on the toxic mechanisms of chemical substances holds the key to developing toxicity prediction methods. For instance, any chemical substance is expected to have hazardous effects on organisms when it is dosed over a threshold level. Strictly speaking, “zero risk” may not be feasible, but safe exposure levels could be set by accumulating quantitative findings on the hazardous properties of chemical substances. Research on the toxicity of chemical substances should be promoted to ensure the safety and security of the public.

Those who have firsthand experience in hazard assessments of chemicals realize clearly that they



require a broad range of knowledge concerning chemical substances such as physico-chemical property, toxicity, fate in the environment and risk abatement techniques, etc. On the international front, the REACH system imposes greater responsibilities on businesses to conduct hazard and risk assessments of chemicals, which is expected to increase the importance of experts in these areas. In Japan, however, a variety of departments such as those of chemistry (Faculty of Science), applied chemistry (Faculty of Engineering), sanitary engineering, veterinary medicine (Faculty of Agriculture), public health (Faculty of Medicine) and pharmaceutical science are engaged independently in education and research on toxicology, which is required for hazard and risk assessments - a situation that may not be ideal for educating and training experts. There is thus a pressing need to set up graduate school in toxicology and its related areas.

In the U.S., some 80 universities offer courses for master and doctoral degrees in toxicology. About 200 students earn a Ph.D. in toxicology every year, and become engaged in research activities at universities, research institutes, companies, administrative agencies and NGOs<sup>[15]</sup>. US society definitely needs toxicology experts (toxicologist). The US government, for instance, is more demanding than its Japanese counterpart as far as chemical substances are concerned; it requires businesses to disclose information regarding the hazardous properties of the chemical substances they handle, along with the amount released into the environment, as a way to respect the public's right to know the truth. In contrast, there are not many toxicologists in Japan. In typical Japanese universities, education and research on toxicology and its related areas are usually conducted by one laboratory in their Faculty of Pharmaceutical Sciences or Medicine. No systematic educational system is in place for toxicology.

"Risk assessment" has become a common buzzword. However, for risk assessments to take root in society, they should be conducted properly, and the public should be able to understand their results correctly. If risk assessments are to be required in Japan, there is a need to improve the institutional framework for

hazard assessments, develop human resources to disseminate information regarding hazard and risk assessments of chemical substances, and train experts to take charge of conducting on-site risk assessments. Graduate schools in toxicology are also expected to play a critical role in developing such human resources.

### Acknowledgements

I would like to express my gratitude to the personnel of the Research Center for Environmental Risk, the National Institute for Environmental Studies, for their support in providing information for this report.

### Notes

- \*1 "Chemical substances" or "chemicals" are such general terms that they could be applied to any material, but for our purposes here they may include not only products sold as chemicals but also unintended byproducts of chemical processes such as dioxin, although those are not included in this report, which only deals with chemicals on the market.
- \*2 A repeated-dose 28-day oral toxicity test costs about ¥7.5-9.5 million and takes about half a year to complete. Long-term toxicity tests are also costly. A typical carcinogenicity test costs about ¥200 million and takes some three years to get results.

### References

- [1] Isono, N. 1975. Chemical Substances and Human Beings, Chuokoron-Shinsha.(in Japanese)
- [2] The ministry of the Environment. Outline of the 1999 Survey of Soil Pollution and Remediation. March 2001. (in Japanese)
- [3] Gamo, M. 2003. Environmental Risk Management Handbook: p130, Asakura Shoten (in Japanese)
- [4] Goka, K., and Urashima, K., "Recent Trends Concerning the Ecological Risk Assessment of Chemicals", p.41 of this bulletin
- [5] The Ministry of Economy, Trade and Industry, the Manufacturing Industries Bureau, the Chemical Management Policy Division, the Chemical Safety Section. Japan

- Chemical Industry Ecology-Toxicology & Information Center, Information A, vol. 25, No.11, 2003. (in Japanese)
- [6] Website of Japan Chemical Industry Ecology-Toxicology & Information Center : <http://www.jetoc.or.jp/excistinfo.html#>
- [7] The 2nd Chemical Substance General Management Policy Workshop, May 2002. : [http://www.meti.go.jp/policy/chemical\\_management/kennkyuukai/kennkyuu-kai-top.htm](http://www.meti.go.jp/policy/chemical_management/kennkyuukai/kennkyuu-kai-top.htm)
- [8] The Ministry of Environment : <http://www.env.go.jp/chemi/seitai-kenko/h13/05/06.pdf>
- [9] Website of OECD; Co-operation in the investigation of existing chemicals : [http://www.oecd.org/departement/0.2688.en\\_2649\\_34379\\_1\\_1\\_1\\_1\\_1.00.htm#top](http://www.oecd.org/departement/0.2688.en_2649_34379_1_1_1_1_1.00.htm#top)
- [10] OECD; Joint meeting of the chemicals committee and the working party on chemicals, pesticides and biotechnology, 36th joint meeting, Feb. 2004.
- [11] Website of the Environmental Protection Agency (US) : <http://www.epa.gov/chertk/volchall.htm>
- [12] Website of EU : <http://europa.eu.int./comm/enterprise/chemicals/chempol/whitepaper/reach/htm>
- [13] Matthews, E.J. and Contrera, J.F. 1998. Reg. Toxicol Pharmacol, 28,242-264.
- [14] Website of NTC : <http://www.niehs.nih.gov/net/home/htm>
- [15] <http://www.gradschool.com/listing/>

(Original Japanese version: published in March 2004)